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A membraneless gas-diffusion unit – multisyringe flow injection spectrophotometric method for ammonium determination in untreated environmental samples

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ABSTRACT

A new design of a membraneless gas-diffusion (MGD) unit coupled to a multisyringe flow injection system is proposed. The spectrophotometric determination of ammonium using an acid–base indicator was chosen to show the feasibility of this approach. Hence, in alkaline medium, ammonium ions are transformed into ammonia (donor channel) which diffuses through the headspace into the acceptor stream (bromothymol blue solution), causing a pH change and subsequently a colour change. The exploitation of the enhanced potentialities of this re-designed MGD device was the main purpose of the present work. Hence, several strategies concerning flow management were studied seeking to characterize and improve the analytical features of the methodology and moreover, untreated environmental samples were analysed without previous filtration. Consequently, stopped flow in acceptor channel with continuous flow in donor channel was chosen for the application to wastewater and spiked river water samples. A linear concentration range between 10.0 and 50.0 mg L^{-1} of NH_4^+, a limit of detection of 2.20 mg L^{-1} and a determination frequency of 11 h^{-1} were obtained.

1. Introduction

Membrane-based approaches for sample pretreatment, namely gas-diffusion (GD) and pervaporation (PV) have proven to be a useful tool when coupled with flow-through techniques, mainly concerning samples with complex matrices. One of the most frequent applications of these approaches among the environmental field is the spectrophotometric determination of ammonium/ammonia using an acid–base indicator [1].

Accordingly, GD combined with flow-based techniques has shown to be effective in the application to the ammonium/ammonia determination in complex environmental samples, such as surface and tap waters [2], marine waters [3], estuarine waters [4], river waters [5] wastewaters [5–8] and compost and fertilizer [9]. However, dirty samples may cause clogging and/or deterioration of the membrane. As an alternative to GD, analytical PV has been explored in order to overcome this drawback by avoiding the contact of sample with the gas permeable membrane. In fact, it relies on the presence of a constant-volume air gap between the sample in the donor chamber and the membrane, which hinders any contact between them [10]. Likewise, GD, the PV technique has also been applied in the analysis of ammonia in wastewaters [11,12]. Nevertheless, a membrane is still necessary to physically separate the sample environment from the acceptor channel.

As follows, a new design of a GD unit, named membraneless gas-diffusion (MGD) unit, was proposed by Choengchan et al. [13]. Unlike the above mentioned units, it allows selective detection of volatile compounds to be made without the need of a hydrophobic membrane. Originally, this device consisted of two pieces: one containing the donor and acceptor channels, parallel to each other and separated by a thin wall with a smaller height, and the other one placed at the top of the former, covering it. Hence, the channels are connected by means of a headspace through which the gaseous analyte diffuses. Thus, besides of the mentioned inconvenience of clogging of the membrane pores when dirty samples are processed, the necessity of frequent changing and the cost of membranes are also overcome. Moreover, without the porous membrane, mass transfer efficiency is greater and, thus, sensitivity is improved. This approach has already been applied, not only to liquid samples [13,14], but also to solids [15,16]. Nevertheless, the membraneless units applied in each case did not have exactly the same configuration as modifications were introduced according to the application aimed. A membraneless gas-diffusion cell (called thin layer distillation cell) was also reported by Mornane and co-workers [17] for the determination of large molecular mass analytes in comparison with PV flow injection separation. It was also investigated

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in a flow injection configuration, although not applied to real samples.

In the present work, the potentialities of a re-designed MGD unit were exploited by using a multisyringe flow injection system (MSFIA). First proposed in 1999 [18], MSFIA has been showing to be a promising flow technique [19,20]. It gathers the advantages of its predecessors flow injection analysis (FIA), sequential injection analysis (SIA), multicommutation flow injection analysis (MCFIA) and successor multipumping flow systems (MPFS). Hence, it has the ability to simultaneously dispense sample and reagents, it offers robustness, versatility, reagent saving and the possibility to establish different sample pathways.

The determination of ammonium in untreated environmental samples (wastewaters and spiked river water samples) was chosen to illustrate the feasibility of this membraneless approach. The methodology chosen was based on the spectrophotometric determination, using an acid–base indicator. Hence, in alkaline medium, ammonium ions are converted into ammonia (donor channel) which diffuses through the headspace into a bromothymol blue stream (acceptor channel), causing a pH change and subsequently a colour change. There are several strategies that can be implemented in order to improve gas transfer efficiency [21,22]. Thus, MSFIA capabilities [19] were employed to study several flow management strategies, besides the study of some physic–chemical parameters, in order to characterize and improve the analytical features of the methodology.

2. Experimental

2.1. Reagents and solutions

Deionized distilled water was used for the preparation of all solutions and chemicals were analytical-reagent grade. The indicator stock solution was prepared by dilution of 0.0646 g of bromothymol blue in 250 mL of water and pH adjustment to 6.5 with a sodium hydroxide solution 0.01 mol L^{-1}. Working solution (8×10^{-5} mol L^{-1}) was prepared by appropriate dilution of the stock solution with water. Sodium hydroxide solutions were prepared by appropriate dilution of a 2 mol L^{-1} stock solution. A 2000 mg L^{-1} stock standard solution of ammonium was prepared by dissolving 1.1863 g of ammonium chloride (dried for 6 h at 100 °C) in 200.0 mL of water. Working standard solutions were prepared daily by appropriate dilution of the stock solution.

2.2. Samples

Influent and effluent samples (including tertiary effluent) of two municipal wastewater treatment plant (D1 and D2), placed at Palma de Mallorca, were collected in January and February 2010 by Emaya laboratory technicians. Table 1 contains the characterization of those samples. Before introduction into the MSFIA system and analysis by the reference procedure (Indophenol blue method) [23], wastewater samples were diluted without previous filtration.

River water samples were collected from two different locations of Douro river (Portugal), at 12 km and 19 km from the shore. A portion of ammonium standard was added to a know volume of sample (river water samples and effluent sample with tertiary treatment) to give a final concentration of 20.0 mg L^{-1} NH_4^+.

2.3. Apparatus

Two multisyringe burettes (Crison Instruments, Allela, Spain, model BU-4-S, 40000 motor steps) were applied, where one propelled solutions (MS 1) to the flow network (*i.e.* MGD unit) and the other one aspirated solutions (MS 0) from it. Both burettes were controlled by computer software: MS 0 was connected through a

Table 1
Wastewater samples characterization (data furnished by Emaya laboratory).

Collection date	Sample	Conductivity (μS cm^{-1} 20 °C)	pH	Turbidity (NTU)	BOD_5 (mg L^{-1} O_2)	COD (mg L^{-1} O_2)	Suspended solids (mg L^{-1})	Total P (mg L^{-1} P)	Total N (mg L^{-1} N)
31/01/2010	D1-EPL	2820	7.75	247	560	1260	724	9.80	73
	D1-TT-SAL	–	8.01	1	1	33	3	5.12	22
	D2-EPL	1958	7.83	208	480	846	244	5.78	61
	D2-SPL	–	–	15	11	99	37	1.25	48
11/02/2010	D1-EPL	2690	–	–	620	900	392	8.65	68
	D2-EPL	2350	7.28	605	900	2320	1348	7.73	70
	D2-SPL	–	–	5	5	524	13	1.46	37
21/02/2010	D1-TT-SAL	–	–	–	–	–	–	–	22

D1-EPL and D2-EPL, influent samples; D1-TT-SAL, effluent samples with tertiary treatment; D2-SPL, effluent sample; BOD_5, biochemical oxygen demand (5 days); COD, chemical oxygen demand.

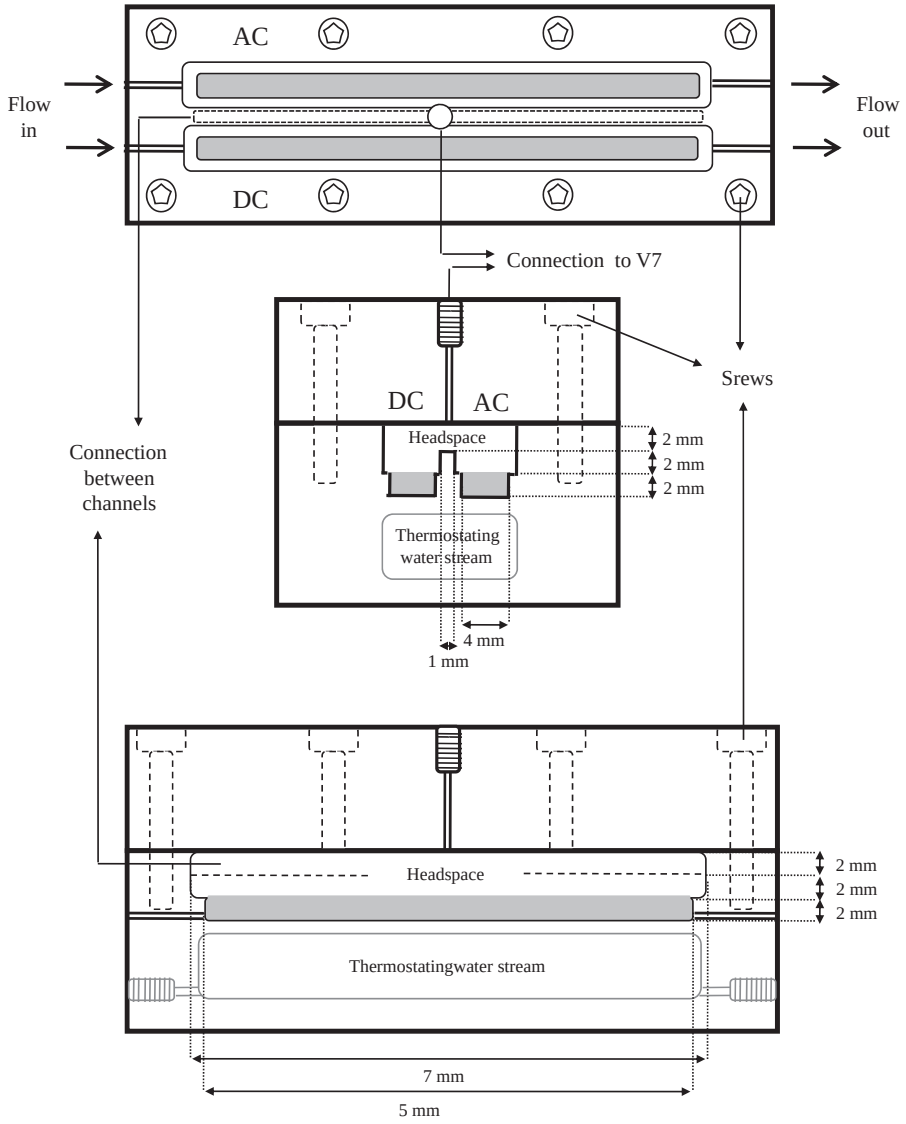

Fig. 1. Membraneless gas-diffusion unit schematic representation through three perspectives: (A) top view; (B and C) lateral views. AC, acceptor channel; DC, donor channel. Gray shadow represents the acceptor and donor streams. For sake of simplicity, PTFE spacer between the two blocks is not shown.

serial port to the computer and MS 1 was connected to the peripheral port of the former. Four syringes (Hamilton, Switzerland), with 5 mL of capacity each, were placed in positions 2 and 3 of MS 0 and MS 1. The head of each syringe was connected to a commutation valve and the manifold was equipped with two extra solenoid valves (Takasago Electric Inc., Nagoya, Japan) in order to allow sample introduction and sample change (V5 and V6). Another extra solenoid valve (V7) was also used, only in specific studies, when an air entrance at the MGD unit was necessary.

An Ocean Optics USB 2000 spectrophotometer (Dunedin, FL) connected to a computer via a USB interface and to a methacrylate cell holder (Sciware, Palma de Mallorca, Spain) accommodating a Hellma 178.712-QS flow cell (18 μL inner volume and 10 mm flow path) and a red LED, with a power supply of adjustable intensity as light source (Sciware), comprised the detection system. The analytical wavelength selected was 635 nm. Instrumental control and data acquisition were accomplished by using the AutoAnalysis 5.0 (Sciware), which is based on dynamic link libraries (DLLs) [24]. Appropriate DLLs were used to operate both multisyringes and the spectrophotometer. MS 1 DLL contemplates the use of the additional solenoid valves.

The MGD unit (Fig. 1) consisted of a homemade device, made of methacrylate and composed by two rectangular blocks: one accommodating two separated channels above of the thermostating water channel ($78 \times 30 \times 28\,mm^3$) and the other acting as lid ($78 \times 30 \times 14\,mm^3$). Both were attached with several screws and separated by a PTFE spacer (1 mm of thickness, rectangular shape, with a central cavity corresponding to the top part of the engraved channels) in order to avoid leakage of gas from the headspace.

For the preliminary studies with different propulsion devices, solenoid micro-pumps (Bio-Chem Valve Inc., Boonton, New Jersey, USA) with a stroke volume of 8 μL (Ref. 090SP12–8) and 20 μL (Ref. 120SP12) controlled by a MCFIA/MPFS module (Sciware, Palma de Mallorca, Spain) and a peristaltic pump (Ismatec SA, Glattbrugg, Switzerland) together with Tygon™ pumping tubes were employed.

2.4. Manifold and MSFIA procedure

All connections of the designed manifold, depicted in Fig. 2, were made of PTFE tubing (0.8 mm i.d.) with lab-made end-

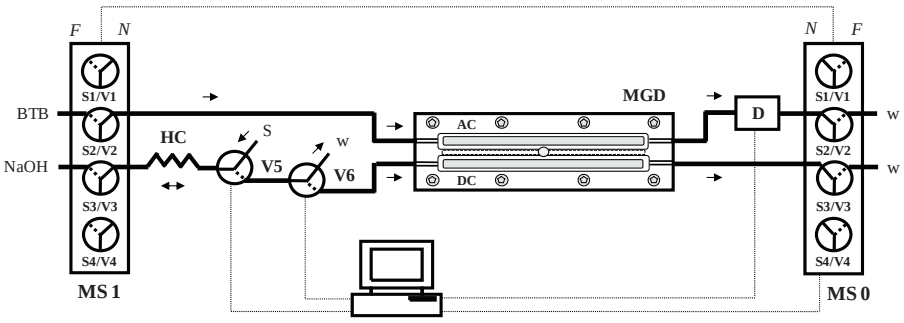

Fig. 2. MSFIA manifold incorporating the MGD unit for the determination of ammonium in wastewaters and spiked river water samples. MS*i*, multisyringe burettes; S*i*, syringes; V*i*, solenoid valves; N, 'on' position (solid line); F, 'off' position (dotted line); HC, holding coil; D, detector; S, standard or sample; W, waste; MGD, membraneless gas-diffusion unit; AC, acceptor channel; DC, donor channel.

fittings and connectors. The holding coil (HC) was 88 cm long.

The determination routine is described in Table 2 and comprises sample aspiration (400 μL) towards the holding coil (steps 1 and 2), followed by continuous flow in donor channel (steps 3–5) while the acceptor stream was stopped, which was subsequently changed to continuous flow in order to be sent for detection (steps 6 and 7). Before each analytical cycle it was necessary to fill the sampling tube with the standard or sample. Hence, 0.300 mL of standard or sample were aspirated towards the holding coil in order to fill the sampling tube and then the excess was discarded through V6 (position 'on') to waste (2000 mL).

3. Results and discussion

3.1. Design of the MGD unit and coupling to MSFIA system

With the aim to exploit the idea of a gas-diffusion unit without membrane, described by Choengchan et al. [13], a similar membraneless unit was constructed following the same dimensions (channels width, 2 mm; dividing wall width, 2 mm; channels depth, 6 mm; dividing wall height, 5 mm; channels length, 5 cm) and shape (inverted U, when side viewed). Further, it was reported that deeper channels solved the cross-contamination problem. Nevertheless, with the similar MGD device described above (NaOH and BTB in donor and acceptor streams, respectively), a capillarity effect was observed as solutions climbed the walls reaching the lid compromising the separation between channels. In order to overcome this limitation, the MGD unit channels were re-designed (Fig. 1) to a "bathtub" form (*i.e.* channels with a border) in order to avoid liquid to stick to the lid, thus breaking the capillary effect. Moreover, channels length was maintained (5 cm), but channels width was increased (4 mm) seeking to facilitate gas-diffusion.

Initial experiments using different propulsion devices coupled to the re-designed MGD unit were performed. At first, a multipumping flow system was designed, however, the pulsed flow showed not to be advantageous in this case. One of the most important parameters when using a MGD unit in a continuous flow mode is that the flow rate at the 'inlet' and at the 'outlet' of donor and acceptor streams must be equal in order to avoid flooding and liquid cross-contamination of the MGD channels [13]. Thus, and due to the difficulty of synchronizing the solenoid micro-pumps action, these pumping devices were abandoned. At the end, the multisyringe was chosen over the peristaltic pump as it avoids the necessity of periodic recalibration of propulsion tubes and moreover, minimizes the reagent consumption.

Thus, a MSFIA system was devised to accommodate the MGD unit using two multisyringes: one propels solutions (MS 1) to the flow network (*i.e.* MGD unit) while another one aspirates solutions (MS 0) from it, simultaneously. As in multisyringe flow systems it is not feasible to introduce the sample into the system by direct filling of one of the available syringes (which would require a large number of washing steps to avoid carry-over between consecutive samples), other devices (*e.g.* selection or solenoid valves) must be incorporated to the manifold to provide access to these solutions. Therefore, solenoid valves V5 and V6 were included in the present manifold to introduce the sample and to perform sample exchange without disturbing the MGD content or the detector, respectively. The volume of sample introduced into the system was defined by the time and flow rate applied during its aspiration [25].

3.2. Study of different strategies to improve analytical features

The main purpose of the present work was to evaluate the MGD unit potentialities for the spectrophotometric determination of ammonium. Hence, sodium hydroxide was used as carrier of sample to allow the conversion of ammonium ions into ammonia (donor stream) and bromothymol blue as indicator (acceptor stream) which, in the presence of ammonia that crossed the headspace, changed its colour, which was further monitored spectrophotometrically. In order to characterize and improve the analytical features of the methodology (*e.g.* sensitivity, liner range, repeatability and determination frequency), several strategies concerning flow management were studied, namely the application of (i) continuous flow in both channels, (ii) flow stop period in both channels and (iii) stopped flow in acceptor channel with continuous flow in donor channel. Working conditions and results concerning each strategy is given in Table 3. Moreover, other strategies were evaluated which are described in subsection (iv). For the following studies the concentration of sodium hydroxide and bromothymol blue (pH 6.5) were fixed at 0.01 mol L^{-1} and 8×10^{-5} mol L^{-1}, respectively.

3.2.1. Continuous flow in both channels

This strategy consisted of dispensing continuously and simultaneously 4 mL of each stream (donor and acceptor) after aspiration of 100 μL of sample.

Under these conditions, the flow rate was varied between 0.3 and 0.7 mL min^{-1}. It was noticed that the lower the flow rate, the greater the sensitivity, but the sampling frequency was worse. The sensitivity achieved for 0.7, 0.6, 0.5 and 0.4 mL min^{-1} was 27, 32, 52 and 75% of that obtained for 0.3 mL min^{-1}, respectively. Furthermore, for the higher flow rates studied, the 4 mL dispensed volume was not sufficient to make the base line return to its initial conditions. With the purpose of rising sensitivity and, thus, lowering the working concentration range, a higher injection volume was tested. Hence, with the flow rate fixed at 0.3 mL min^{-1} and aspirated sample volume at 400 μL, the sensitivity was enhanced 2.2-fold, enabling determination at a concentration range between 5.0 and 50 mg L^{-1} (instead of 50 and 150 mg L^{-1}), with a good

Table 2
Protocol sequence for the ammonium determination using the MGD unit coupled to MSFIA.

Step	Multisyringe MS 1							Multisyringe MS 0					Description
	Volume (mL)[a]	Flow rate (mL min $^{-1}$)	Piston movement	SV position[b]				Volume (mL) [a]	Flow rate (mL min $^{-1}$)	Piston movement	SV position[b]		
				V2	V3	V5	V6				V2	V3	
1	0.100	15	Pick up	F	F	F	F	–	–	–	–	–	Dummy step
2	0.400	1.5	Pick up	F	N	N	F	–	–	–	–	–	Sample is aspirated
3	0.100	15	Dispense	F	F	F	F	–	–	–	–	–	Dummy step
4	2.400	0.9	Dispense	F	N	F	F	2.400	0.9	Pick up	F	N	Stopped flow in AC with continuous flow in DC
5	0.600	15	Pick up	F	F	F	F	1.500	15	Dispense	F	F	Syringes are positioned
6	3.000	2.0	Dispense	N	N	F	F	3.000	2.0	Pick up	N	N	Detection and data acquisition
7	4.400	15	Pick up	F	F	F	F	3.900	15	Dispense	F	F	Syringes are repositioned

AC, acceptor channel; DC, donor channel.
[a] Initially syringes were filled up to 88% (MS 1) and 2% (MS 0) of their capacity.
[b] SV, solenoid valve; N and F represent position 'on' and 'off', respectively.

Table 3
Working conditions and figures of merit concerning the flow management strategies studied.

Flow management strategy	Variable studied	Fixed conditions	Sensitivity (AU mg^{-1} L NH_4^+)	Concentration range (mg L^{-1} NH_4^+)	RSD (%) $n = 10$ (25 mg L^{-1} NH_4^+)	Determination frequency (h^{-1})
Continuous flow in both channels	Flow rate (mL min^{-1})	Sample volume (mL)				
	0.3		0.00740	50–150		
	0.4		0.00554			
	0.5	0.100	0.00388			
	0.6		0.00236			
	0.7		0.00202	50–200		
Flow stop period in both channels	Flow rate (mL min^{-1})	Sample volume (mL)				
	0.3	0.400	0.0132	5.0–50	4.6	4.3
	Sample volume (mL)	Stop period (s)				
	0.100	300	0.00783	5.0–50		
	0.200		0.0130	5.0–50	5.8	7.2
	Sample volume (mL)	Stop period (s)				
	0.200	450	0.0153	5.0–50		
Stopped flow in acceptor channel with continuous flow in donor channel	Sample volume (mL)	Flow rate (mL min^{-1})				
	0.100		0.00228	5.0–50		
	0.200	0.3	0.0125	5.0–50		
	0.400		0.0296	2.0–25		
	Flow rate (mL min^{-1})	Sample volume (mL)				
	0.5	0.400	0.0182	5.0–50	4.8	8.0
	0.7		0.0110	5.0–50		

All these experiments were performed with BTB 8×10^{-5} mol L^{-1}, pH 6.5 (acceptor channel) and NaOH 0.01 mol L^{-1} (donor channel).

repeatability (4.6%), but with deprived determination frequency (4.3 h^{-1}).

3.2.2. Flow stop period in both channels

The analytical procedure for this study comprised the introduction of the mixture NaOH/sample containing ammonium within the MGD device, followed by a stop period in both channels. Thus, after sample introduction into the holding coil, and by flow reversal, 200 μL of NaOH/sample was sent towards the MGD unit to place the mixture at its entrance (while acceptor stream was stopped). Then, solenoid valve V7 was activated to allow the withdrawal of the donor channel (using only MS 0, syringe S3) before filling it with the NaOH/sample mixture (using only MS 1, syringe 3), which was followed by a stop period. Then, 3 mL of acceptor and donor stream were sent simultaneously towards detection and waste, respectively, at 2.0 mL min^{-1}.

Using this protocol sequence and a stop flow period of 300 s, the sample volume was tested. Due to the limit imposed by the dimensions of the membraneless unit channels, a maximum sample volume of 200 μL was placed within the donor channel. The sensitivity achieved for 100 μL was 60% of that obtained for 200 μL. Setting the sample volume at 200 μL, a stopped flow period of 450 s was also tested, although sensitivity was only enhanced 1.2-fold. Thus, with the previous conditions (sample volume of 200 μL and stopped flow period of 300 s), a better determination frequency was achieved when compared to that attained by the former strategy, but with a lower repeatability (5.8%).

3.2.3. Stopped flow in acceptor channel with continuous flow in donor channel

To characterize this strategy, the subsequent procedure was adopted: sample aspiration into the holding coil, followed by continuous flow in donor channel (dispensing 2.4 mL from MS1, syringe 3) while the acceptor stream was stopped. Afterwards, 3 mL of acceptor and donor stream were sent simultaneously towards detection and waste, respectively, at 2.0 mL min^{-1}.

With the flow rate set at 0.3 mL min^{-1}, the sample volume was studied, and the sensitivity achieved for 100 μL and 200 μL was 8 and 42% of that obtained for 400 μL, respectively. Fixing the sample volume at 400 μL, the flow rate was varied. Hence, the sensitivity attained for 0.5 mL min^{-1} and 0.7 mL min^{-1} was 61 and 37% of that obtained for 0.3 mL min^{-1}, respectively. A repeatability of 4.8%, a determination frequency of 8 h^{-1} and a working concentration range between 5.0 and 50 mg L^{-1} were achieved with a flow rate of 0.5 mL min^{-1} and sample volume of 400 μL. This strategy was chosen for further application as these analytical features fitted our interest towards wastewater samples.

3.2.4. Other strategies to improve analytical features

As mentioned above, the strategy based on stopped flow in acceptor channel with continuous flow in donor channel was chosen for the application to wastewater samples. Nevertheless, to ensure the complete transformation of ammonium ions into ammonia, a solution of 0.1 mol L^{-1} NaOH was selected instead of 0.01 mol L^{-1}. As raising the NaOH concentration decreased the linear range, the flow rate was assessed once again to set the final conditions. Hence, the sensitivity obtained for 0.7 and 0.9 mL min^{-1} was 60 and 25% of that achieved for 0.5 mL min^{-1}. A flow rate of 0.9 mL min^{-1} was chosen as an acceptable compromise between the speed of analysis and the target concentration range within 10.0–50.0 mg L^{-1}.

However, if samples with lower concentration are to be analysed (*e.g.* river or sea water samples), sensitivity must be greater in order to lower significantly the concentration range. Hence, other parameters were studied, such as BTB volume in the acceptor channel,

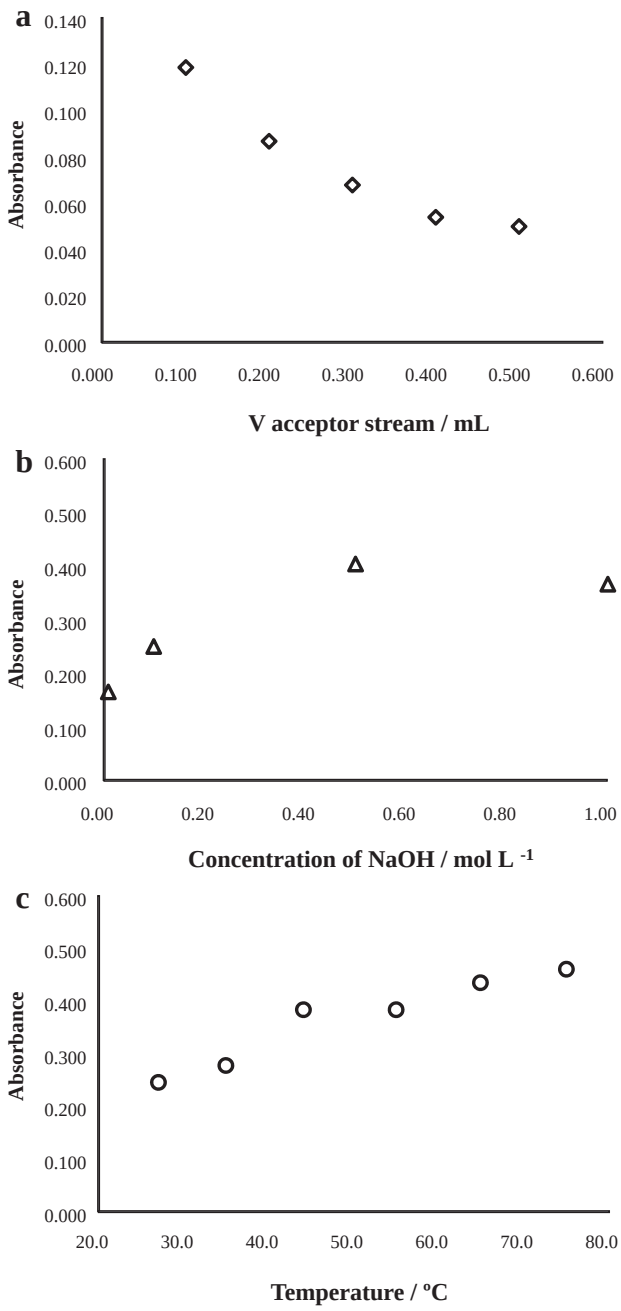

Fig. 3. Influence of the (a) acceptor stream volume, (b) NaOH concentration and (c) temperature on the absorbance obtained when analysing 5.0 mg L^{-1} of NH_4^+. Experimental conditions: (a) NaOH concentration, 0.01 mol L^{-1}; donor channel flow rate, 0.5 mL min^{-1}; sample volume, 400 μL; (b) acceptor stream volume, 0.200 mL; donor channel flow rate, 0.3 mL min^{-1}; sample volume, 400 μL; (c) acceptor stream volume, 0.200 mL; donor channel flow rate, 0.3 mL min^{-1}; sample volume, 400 μL; NaOH concentration, 0.5 mol L^{-1}.

NaOH concentration and temperature, by assessing the absorbance obtained when analysing 5.0 mg L^{-1} of NH_4^+.

BTB volume within the acceptor channel was a critical aspect because we observed that ammonia retention took place at the surface of the liquid filling the acceptor channel. When enough acceptor solution was present, two coloured layers were observed, a lower yellow layer corresponding to acidic BTB and a top blue layer corresponding to basic BTB due to ammonia adsorption. Hence, BTB volume was studied in order to minimize the dilution effect fostered by the lower layer of unreacted BTB. Volumes between 0.100 and 0.500 mL were studied, as shown in Fig. 3a. The

volume of 0.100 mL was not sufficient to cover the bottom surface of the acceptor channel, as occasionally air bubbles passed through the detector. Nevertheless, this did not occurred with the remaining tested volumes and, thus, the signal obtained for a BTB volume of 0.500 mL was 57% of that obtained for 0.200 mL.

Hence, choosing 0.200 mL of BTB volume in the acceptor channel and lowering the flow rate of the donor stream from 0.5 to 0.3 mL min^{-1}, the concentration of NaOH was varied between 0.01 and 1.0 mol L^{-1} (Fig. 3b). The analytical signal was improved up to 0.5 mol L^{-1} NaOH. Above this value, the signal was rather maintained. Thus, by raising the NaOH concentration from 0.01 to 0.5 mol L^{-1}, the analytical signal was enhanced 2.4-fold. Therefore, the chosen concentration for the next assessment was 0.5 mol L^{-1}.

The temperature effect was studied between 27 and 75 °C (Fig. 3c), by flowing water at the mentioned temperatures through the thermostating water stream (Fig. 1b and c), in order to improve ammonia vaporization. The absorbance obtained for 27, 35, 44, 55 and 65 °C was 54, 61, 83, 84 and 95% of that obtained for 75 °C. Condensation development at the lid surface was noticed for the highest temperatures and it also affected repeatability for 75 °C. Accordingly, by selecting 65 °C of temperature, a sensitivity of 0.0940 was possible to attain enabling a concentration range between 1.0 and 4.0 mg L^{-1}. The possibility of heating only the donor channel instead of both channels was also considered, although not tested. Even if a narrow thermostating water stream would be created below the donor channel in alternative, due to the proximity among channels it would not be possible to avoid some heat transference to the acceptor stream.

3.3. Figures of merit

Under the chosen conditions for the application to wastewater samples (Table 4), a linear response between absorbance and ammonium concentration was obtained over the range of 10.0–50.0 mg L^{-1} NH_4^+.

The limit of detection for the proposed methodology was 2.20 mg L^{-1} NH_4^+, calculated as the concentration corresponding to the blank signal plus three times the standard deviation of ten consecutive blank injections. The blank signal was obtained by injecting a solution with the same composition as the standards, except for the ammonium.

The repeatability of this system was assessed from ten injections of ammonium standard 20.0 mg L^{-1} NH_4^+. The corresponding relative standard deviation values were 4.8%.

The determination frequency was estimated as the sum of the time needed for each step of the analytical cycle and the time required for data transference between the computer and the multisyringes. A complete analytical cycle took 358 s: 322 s for each determination plus 36 s necessary when the sample was switched. Thus, the determination frequency was 11 per hour and the sample frequency was about 4 per hour considering three replicate determinations for each sample.

For comparison purposes, Table 4 contains the figures of merit of the proposed MSFIA system coupled to the MGD device and of different flow systems coupled to GD and PV units for the determination of ammonium in environmental samples. Through its analysis we can emphasize that the most remarkable feature of the methodology herein described is the absence of membrane, which allows analyzing directly untreated samples and reducing the cost per analysis. In all of the presented examples, except one [11], samples needed at least to be filtered before introduction into the flow system. Moreover, the membrane needed to be replaced every month as a minimum. These procedures are tedious and time-consuming, time which is not contemplated by the determination frequency parameter. Even when the PV approach is applied, sample pretreatment can be necessary [12]. Similar analytical features

Table 4
Figures of merit of the proposed MGD-MSFIA system and of membrane-based flow systems described in the literature for the determination of ammonium in environmental samples.

Flow system/separation technique	Linear range (mg L^{-1} NH_4^+)	LOD (mg L^{-1} NH_4^+)	RSD (%)	Determination throughput (h^{-1})	Reagent consumption (per determination)	Sample treatment before flow analysis	Membrane lifetime
FIA/GD [6]	0.5–60	0.5/1	<2.5	NA	NA	Filtration	1 day/1 month
FIA/GD [7]	1.29–129	0.77	3.4	12	0.5 mg NaOH; 0.17 mg BTB	Filtration	1 week
SIA/GD [8]	2–60	2	2.5	NA	0.04 mg NaOH; 0.13 mg BTB	Filtration	Several months
MCFIA/GD [2]	0.05–1	0.03	1.5	20	10.5 mg NaOH; BTB re-circulation	Filtration	2 weeks
MSFIA/GD [9]	5–70	0.03	1.3	20	0.1 mg NaOH; 0.2 mg BTB	Dissolution, extraction, filtration	NA
MPFS/GD [5]	0.3–5.0	0.02	<1.2	50	0.03 mg $NaClO_2$; 0.1 mg luminol	Filtration	NA
FIA/PV [11]	0.2–20	0.1	3	11	150 mg NaOH; 0.04 mg CR; 0.1 mg TB	No treatment	1 month
FIA/PV [12]	0.05–50	0.03	1.9	8/10	NA	Filtration	NA
MSFIA/MGD (present work)	10–50 (2.0–200)[a]	2.2	4.8	11	200 mg NaOH; 0.2 mg BTB	No treatment	No membrane

BTB, bromothymol blue; CR, cresol red; FIA, flow injection analysis; GD, gas-diffusion; LOD, limit of detection; MCFIA, multi-commuted FIA; MGD, membraneless GD; MPFS, multi-pumping flow system; MSFIA, multisyringe FIA; NA, not available; PV, pervaporation; SIA, sequential injection analysis; TB, thymol blue.

[a] A concentration range between 2.0 and 200 mg L^{-1} is attainable according to the flow management strategy used (together with other variables).

Table 5
Results obtained by MSFIA methodology (C_{MSFIA}) and by the reference method (C_{RM}) for the determination of ammonium in wastewater samples. Relative deviations (R.D.) are also given.

Samples	C_{MSFIA} (mg L^{-1} NH_4^+)	C_{RM} (mg L^{-1} NH_4^+)	R.D. (%)
D1-EPL[a]	80.6 ± 2.7	80.6 ± 2.1	0.0
D2-EPL[a]	64.0 ± 1.8	63.7 ± 6.2	0.5
D2-SPL[a]	53.3 ± 2.9	54.1 ± 4.3	−1.5
D1-EPL[b]	70.2 ± 2.4	69.4 ± 1.8	1.2
D2-EPL[b]	73.6 ± 6.6	72.7 ± 0.6	1.2
D2-SPL[b]	50.2 ± 6.5	50.1 ± 1.3	0.2

[a] Collection date: 31/01/2010.
[b] Collection date: 11/02/2010.

where obtained when compared with the selected FIA [6,7,11,12] and SIA [8] systems (*viz.* linear range, determination throughput), though presenting more robustness as it avoids the use of peristaltic pumping tubes commonly used in FIA. Lower linear range and limit of detection where attained by the MCFIA [2] and MPFS [5] examples. However, the multisyringe burette consists of a more robust propulsion device for the reason mentioned above and additionally because it does not require frequent recalibration (especially when compared with micro-pumps). In relation to reagents consumption, though the amount of NaOH consumed by the present methodology is rather higher than the other flow systems, the amount of BTB is similar.

3.4. Application to wastewaters and spiked river water samples

After establishing the working conditions, the system was applied to analysis of wastewater (characterized in Table 1) and spiked river water samples. Six wastewater samples obtained from wastewater treatment plants, were analysed by the proposed MSFIA procedure (C_{MSFIA}) and by the usual reference method (C_{RM}) [23]. The results, together with the corresponding relative deviations, are presented in Table 5. To evaluate the accuracy of the developed methodology, a linear relationship ($C_{MSFIA} = S \times C_{RM} + C_0$) was established, including all the mentioned samples. Hence, $C_{MSFIA} = 1.03\ (\pm 0.065)\ C_{RM} + 1.5\ (\pm 4.3)$, $R = 0.999$ was obtained ($n = 6$), where the values within parentheses are 95% confidence limits [26]. Considering these values, we concluded that the estimated slope and intercept do not differ significantly from 1 and 0, respectively. Therefore, there is no evidence for systematic differences between the sets of results obtained by the proposed methodology and those obtained by the reference method. A paired *t*-test was also applied. The calculated *t* (0.859) was lower than the critical *t* value (2.57, $p = 0.05$, *d.f.* = 5), which also indicates the absence of systematic differences between the results of the two methods [26]. Recovery assays in two wastewater samples (tertiary effluents) and two river water samples were also performed. Recovery values close to 100% were achieved with an ammonium concentration added to all the mentioned sample of 20.0 mg L^{-1} NH_4^+ (Table 6). By examining the wastewater samples characterization in Table 1, it can be observed that the ammonium values obtained with the proposed MSFIA method follow the same tendency as those from other parameters: the values presented in the samples collected before the treatment plant are, as expected, higher than the ones collected after it.

4. Conclusions

The proposed MSFIA system coupled to the re-designed MGD device, showed to be suitable for the determination of ammonium in complex environmental samples, with no previous filtration step. By taking advantage of MSFIA versatility, several strategies concerning flow management were studied in order to enhance the analytical features. Thus, a wide concentration range (2.0–200 mg L^{-1} NH_4^+) was possible to attain without any physical reconfigurations, as parameters where changed through software control. Nevertheless, for the application in question, stopped flow in acceptor channel and continuous flow in donor channel was the chosen strategy.

Table 6
Results obtained (mg L^{-1} of NH_4^+) in recovery studies using effluents with tertiary treatment (D1-TT-SAL) and river water samples.

Samples	Added	Found[e]	Recovery[e] (%)
D1-TT-SAL[a]	0	<LOD	–
	20.0	21.8 ± 0.1	109 ± 0
D1-TT-SAL[b]	0	<LOD	–
	20.0	18.3 ± 0.9	91.5 ± 4.6
River water[c]	0	<LOD	–
	20.0	19.3 ± 1.2	96.4 ± 5.8
River water[d]	0	<LOD	–
	20.0	20.6 ± 0.5	103 ± 2

[a] Collection date: 31/01/2010.
[b] Collection date: 21/02/2010.
[c] Collected at 12 km from the shore.
[d] Collected at 19 km from the shore.
[e] $n = 3$.

The selected spectrophotometric method consists of an environmentally friendly alternative to the indophenol blue method, avoiding the use of toxic reagents. Furthermore, in comparison to flow systems comprising membrane-based devices (*viz.* GD and PV) with application to environmental samples (Table 4), similar results could be obtained (*i.e.* concentration range, determination frequency, BTB consumption). Although, additional advantages are provided: the present methodology is less expensive, due to the absence of membrane, allows the direct introduction of untreated samples and is more robust as it avoids the use of peristaltic pumping tubes or solenoid micro-pumps commonly used in FIA/MCFIA and MPFS, respectively.

In fact, the proposed system could not reach ammonium concentrations below 1.0 mg L^{-1} NH_4^+. Nevertheless, good recoveries were achieved for samples with low levels of ammonium (wastewaters and river water samples). Hereafter, the analytical performance of the MGD unit could be improved through the arrangement of its headspace volume or channels profile.

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